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WORLD REFERENCE CENTER FOR ARBOVIRUSES AND RETROVIRUSES

ANNUAL REPORT

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MAY 1, 1988

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Grant No. DAMD17-87-G-7005

Yale University School of Medicine
New Haven, Connecticut 06510

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Yale University School of Medicine; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Box 3333 New Haven, Conneticut 06510	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION: U.S. Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-87-G-7005
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick Frederick, Maryland 21702-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 61102A; PROJECT NO. 3M1-61102BS13; TASK NO. AA; WORK UNIT ACCESSION NO. WUDA312093

11. TITLE (Include Security Classification)

(U) World Reference Center for Arboviruses and Retroviruses

12. PERSONAL AUTHOR(S)

Robert E. Shope

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 2/9/87 TO 2/8/88	1988 May 1	50

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Arbovirus, serological diagnosis, ELISA, complement-fixation, orbivirus, monoclonal antibodies, Cache Valley virus, Japanese encephalitis virus, dengue virus, RA 1
06	13		
06	03		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The World Reference Center for Arboviruses and Retroviruses identified viruses from Thailand, Nepal, Egypt, Colombia, and Panama. Cache Valley virus from a recruit on jungle training was characterized. RNA-RNA hybridization of orbiviruses and reassortment of these dsRNA viruses correlated. Attempts to identify by IFA a novel agent from Kaposi's sarcoma cases were negative with a battery of arboviruses; retrovirus (HIV) isolation capability was transferred to Bogota in a collaborative study. Virus reference reagents for arboviruses were distributed to military laboratories and other laboratories world-wide. A low passage collection of viruses was augmented to over 330 strains.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION	
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mary Frances Bostian	22b. TELEPHONE (Include Area Code): 301-663-7325	22c. OFFICE SYMBOL: SGRD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

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SUMMARY

Studies of two serogroups in the genus Orbivirus led to a proposal for defining species. The correlation between the degree of RNA-RNA hybridization and genetic reassortment of these double stranded RNA viruses was excellent. RNA-RNA hybridization was an efficient marker of geographic origin of Kemerovo serogroup viruses and confirmed the Siberian origin of Kemerovo virus isolated from a migrating bird in Egypt.

Viruses were identified from Thailand, Nepal, Egypt, and Colombia. Eight new phleboviruses were characterized serrologically. A virus isolated from blood of a febrile U.S. Army recruit on jungle training in Panama was identified as a subtype of Cache Valley virus.

Monoclonal antibodies were developed and characterized for vesicular stomatitis (VSV), Indiana, Cocal, and Alagoas; and for Cache Valley viruses. The VSV antibodies were used to type Indiana virus subtypes.

Semliki Forest virus monoclonal antibodies were used to find a conserved epitopic region on the alphavirus nucleocapsid protein.

Cache Valley virus was associated with an epizootic in sheep of arthrogryposis and hydranencephaly affecting nearly 20% of offspring in Texas and Nebraska. This is the first time a bunyavirus has been implicated in this disease in the Americas.

Attempts to identify by IFA a novel enveloped virus isolated from tissues of patients with Kaposi's sarcoma were negative with a large battery of arboviruses. Retrovirus (HIV) isolation capability was transferred to Bogota in a collaborative study.

Japanese encephalitis trpE fusion proteins were used as highly specific diagnostic reagents in ELISA. These proteins were not very immunogenic to mice. Primer extension sequencing of the NS1 region of the genome yielded epidemiologic information indicating there is geographic clustering of dengue-1 strains.

The low-passage arbovirus collection now contains in excess of 330 strains of chikungunya, VSV, California encephalitis group, Venezuelan encephalitis, Japanese encephalitis, dengue, and other arboviruses pathogenic to man and domestic animals.

As in the past, the world Reference Center for Arboviruses distributed viruses, antigens, antibodies, cell cultures, and colonized arthropods to laboratories, both in the United States and world-wide.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

For the protection of human subjects, the investigators have adhered to policies of applicable Federal Law 45CFR46.

BODY OF REPORT

I. ARBOVIRUS TAXONOMY

A. Orbivirus speciation: Genetic reassortment between Corriparta serogroup viruses (H.A. Gonzalez and D.L. Knudson). The question of what constitutes a species in virology has not been answered. One approach is to adopt the biological definition of Mayr in which a species is "a reproductive community of populations that occupies a specific niche in nature." The orbiviruses offer an ideal model because orbiviruses have 10-12 RNA segments which can reassort and thus they present an analogy to sexual reproduction in other organisms.

In these experiments, the ability of orbiviruses of the Corriparta serogroup to reassort genes in vitro was examined. Table 1 shows the wide distribution of the members of the serogroup in time and geographic location. Eight members were used in this study. These are all cross-reactive by CF, but exhibit distinct electropherotypes by 10% polyacrylamide gel electrophoresis (PAGE) and are distinguishable by RNA-RNA blot hybridization. The differences in PAGE electropherotypes permitted recognition of reassortant viruses.

Viruses were grown in BHK-21 cells, and BHK-21 cell plaque assays were used to titrate viruses and to plaque possible reassortant viruses. The in vitro reassortment experiments were done in 96-well microplates seeded at 10,000 cells per well and incubated overnight. Then mixtures of viruses using different MOI's of each virus to vary the mixed-ratio were adsorbed to the monolayer. Each virus in the mixture was titrated. After 24 h incubation of the mixtures, the progeny were harvested, plaqued, and selected populations tested for reassortants by PAGE analysis of the dsRNA.

The results of the in vitro reassortment experiments are shown in Table 2. Reassortment was observed between each pair of viruses crossed. The ratio of progeny viruses was affected directly by the initial MOI ratio because an increase in the proportion of one parent resulted in an increase in the proportion of progeny viruses with a genotype indistinguishable from the parent. For example, the higher MOI of CSIRO 76 in Jacareacanga X CSIRO 76 crosses resulted in an increase in the proportion of CSIRO 76 in the progeny (Table 2).

Since some segments from different viruses comigrate in the RNA gels, the actual number of reassortants resulting from a cross represents a conservative estimate, and it may be greater than or equal to the observed number.

The observed geographic distribution of the Corriparta serogroup isolates and hybridization data suggest that at least three distinct allopatric populations exist among the eight isolates. Yet, viruses in the three subsets represent a common gene pool in which genes are exchanged even though geographic barriers to their genetic interaction exist and must be removed. Only isolates circulating in the same locality would have an opportunity to reassort in nature. For example, the Australian isolates originated in the same locality and were isolated from the same vector species, implying that reasortment between these isolates could occur either in the mosquito vector or vertebrate host. Similarly, reassortment

between the two Brazilian isolates may also occur in nature. The two remaining isolates, Acado and Bambari, may represent populations which are reproductively isolated by geography.

The in vitro reassortment demonstrated here reflects the common ancestry of the Corriparta serogroup viruses, and suggests that all eight isolates should be considered members of a single species. This serves as a model for other Orbivirus serogroups in which gene conservation is demonstrated by RNA-RNA blot hybridization and which may be single species.

Table 1

Corriparta serogroup viruses used in reassortment experiments

Virus isolate	Source	Geographical origin	Date of collection
Corriparta			
MRM 1	Cu. annulirostris	N. Queensland, Australia	3/30/60
CSIRO 76	Cu. annulirostris	N. Territory, Australia	9/03/75
CSIRO 109	Cu. annulirostris	N. Territory, Australia	6/11/75
CSIRO 134	Cu. annulirostris	N. Territory, Australia	4/23/75
Bambari			
DakArB3689	Culex pool	Bambari, Centr. Afr. Republic	11/22/71
BeAr263191	Cu. declarator	Belem, Brazil	11/11/74
Jacareacanga			
BeAr295042	Cu.(Mel.) spp.	Belem, Brazil	11/11/75
Acado			
EthAr1846-64	Cu. antennatus & Cu. univittatus naevi	Ilubabor, Ethiopia	10/22/63

Table 2

In vitro reassortment of Corriparta serogroup viruses

Parental cross (F1 x P2)

minimum number of segments distinguishable	MOI ratio (PFU/cell) (P1:P2)	observed progeny (P1:P2: reassortant)	reassortment genotype (segment 1 2 3 4 5 6 7 8 9 10
CS 109 (1) x CS 76 (7) 5	12:9	2:14:4	X X 7 1 7 1 7 7 X 7*
			X X X 7 7 1 7 X X 7
			X X X 1 7 7 7 X X 7
			X X X 1 7 1 7 X X 1
Corriparta (C) x CS 76 9	10:7	4:4:1	7 7 C 7 C C C 7 7 7
	7:7	2:2:3	C 7 7 C C 7 7 7 X 7
			7 7 C C C C C 7 X 7
			7 7 7 7 7 C 7 7 X 7
Corriparta x Acado (A) 10	50:3	9:0:1	C A C C A A A A A C
Jacareacanga (J) x Acado 6	26:29	5:9:4	X J J J J J J X J A
			X J J X X J A X J A
			X J J X X J J X J A
			X A J X X A A X A A
Jacar. x BeAr263191 (2) 8	15:15	7:0:3	2 J J J J 2 X J J 2
			J J 2 J J J 2 X X J
			2 J J J J J X J J J
Corriparta x BeAr263191 10	27:12	8:0:2	2 C C 2 C 2 2 2 C C
			2 2 2 2 C 2 2 2 2 2
Jacar. x Corriparta 8	15:19	8:0:2	J C J J J J J J C J
			J C J C X J J J X J
Jacareacanga x CS 76 7	5:42	0:8:2	J 7 J J J X X 7 X J
			7 7 J 7 7 X X 7 X 7
	15:14	9:0:1	J 7 J J J X X 7 X 7
Acado x CS 76 8	13:7	4:4:2	A A 7 A A A X A A X
			7 7 7 A 7 A X 7 7 X
Acado x Bambari (B) 5	58:36	5:5:2	A X A B A X A A X X
			X X A B A X A A X X
Bambari x Jacareacanga 9	4:8	5:2:3	J J J B J J J J B J
			J X J J J J J B B B
			J X J J J J J B B J
	18:26	7:3:3	J X J J J J J J J B
			B X B J J B B B B J
			B X J B J B B B B J

*The parental origin of each segment is indicated by a single letter or number abbreviation; segments of undetermined origin are indicated by letter X.

B. Genetic reassortment and RNA-RNA hybridization of Kemerovo serocomplex viruses (S.E. Brown, H.G. Morrison, S.M. Buckley, R.E. Shope, and D.L. Knudson).

The taxonomy of the Kemerovo serogroup viruses has been revised using RNA-RNA hybridization and genetic reassortment. Casals proposed a classification in which the Kemerovo serogroup was divided into three serocomplexes. The Chenuda, Kemerovo, and Seletar (Wad Medani) complexes were distinguished by complement-fixation (CF) tests. Main later defined a fourth serocomplex, the Cape Wrath-Great Island complex. This complex now contains the largest number of members. All viruses in the serogroup are transmitted by either argasid or ixodid ticks.

The Kemerovo serocomplex contains the Ixodes persulcatus tick and human isolates made by Soviet scientists in Western Siberia in 1962 and the isolate from a migrating redstart by Schmidt in 1961. These are serologically indistinguishable. Additional subtypes including Tribec, distinguishable from the Soviet viruses by neutralization tests, were isolated in Czechoslovakia in 1963 from Ixodes ricinus and small mammals.

These Kemerovo serocomplex viruses, listed in Table 3, were examined by RNA-RNA blot hybridization and gene reassortment to determine their sequence and functional relatedness.

Viral suspensions containing sets of two parental virus stocks in varying multiplicity of infection (m.o.i.) ratios were prepared. BHK-21 cells were inoculated, the virus mixtures incubated at 32C for 24 h, and then the progeny titrated in plaque assays. The plaques were picked and the resulting progeny examined by polyacrylamide gel electrophoresis (PAGE) to determine which parent virus had donated each segment. Some of the viruses were also electrophoresed in agarose. The dsRNA of selected reassortants was end-labeled with 32P and the segments analyzed by PAGE.

RNA probes were prepared by purification of dsRNA and then 3' end-labeling with 40 uCi of [5'-32P] pCp. The RNA was electrophoretically transferred and hybridized at Tm(RNA)-36.

Agarose profiles of selected dsRNA genomes of the Kemerovo serocomplex viruses were similar. In contrast, these isolates exhibited unique PAGE dsRNA profiles. While the PAGE dsRNA profiles were distinct, the profiles of the Czechoslovakian isolates were more heterogeneous than the Siberian and Egyptian isolates. The original Tribec (Czechoslovakia) isolate contained several genetic types (or genotypes) as evidenced by the appearance of twelve segments in its dsRNA PAGE profile. This virus stock was plaque-purified, and four different genotypes of 10 dsRNA segments were found.

The EgAn 1169-61 (Egypt) probe hybridized strongly to the Siberian isolates, and it hybridized to a lesser extent with the Czechoslovakian isolates. In contrast, the Tribec probe hybridized weakly to members of the Siberian subtype isolates and strongly to the Czechoslovakian isolates. One isolate from Czechoslovakia, Lip 53, was variant in gene 4 with respect to the Tribec probe. In reciprocal hybridizations, gene 3 among the Siberian and Czechoslovakian subtype isolates hybridized with a weaker signal than did the other genes. The labeled Tribec probe (clone 1) was

chosen to represent the predominant genotype in the Tribec virus population. The four clones of Tribec virus exhibited high conservation in their sequence by hybridization.

Fifty per cent of the progeny from the mixed infection (or cross) of the two Siberian serotype isolates, L75 and EgAn 1169-61, were reassortants (Table 4), and 67% of the progeny from the cross between the two Czechoslovakian isolates, Tribec (clone 1) and Kol 152, were reassortants (Table 4). Gene reassortment was also demonstrated between the two isolates representing the Czechoslovakian and Siberian subtypes. A difference was observed in the progeny from the cross between the Siberian isolate, L75, and the plaque-purified Czechoslovakian isolate, Tribec. For example only eight reassortants (9%) were observed in progeny from this cross, and seven were single gene replacements. In contrast, more than half of hte reassortant progeny from intra-typic crosses had multiple gene replacements (Table 4). While gene reassortment was demonstrated in both intra- and inter-typic crosses, the inter-typic reassortment yielded fewer reassortant progeny with only a few gene substitutions.

Several observations are evident from this study:

a. Isolates within each subtype of the Kemerovo complex are generally indistinguishable by serologic tests, and by blot hybridization with one exception. Lip 53 was the only isolate which was distinguishable within the Czechoslovakian isolates by blot hybridization because its gene 4 was variant when compared to the other isolates. Nevertheless, the distinct dsRNA PAGE profiles for both the Siberian and Czechoslovakian subtypes were indicative of genetic drift in both sybtypes, with members of the Siberian subtype being relatively more homogeneous when compared to the Czechoslovakian isolates.

b. While the genes of Tribec virus were conserved among the four plaque-purified clones by blot hybridization, the presumed presence of the different genotypes in the original virus stock may explain a relatively broad antigenic structure commented on for Tribec in CF tests by Libikova in her 1971 publication. These data suggest that at least two genotypes were co-circulating in the Tribec region.

c. In these hybridization experiments, light signals identified variant genes. At Tm(RNA)-36 hybridization conditions, sequences among variant genes approached the lower limit of 74% homology required for the formation of stable hybrids. While the Czech and Soviet subtypes cross-hybridized in all ten genes, the existence of light signals indicated that the sequence relatedness between the two subtypes approached the lower limit of 74% sequence homology.

d. Although the EgAn 1169-61 (Egypt) virus was isolated from a migratory redstart captured in Egypt in 1961, this isolate was indistinguishable from the Siberian isolates by serology, blot hybridization, and gene reassortment. The redstart appears to have acquired the virus in Siberia and this suggestion is consistent with ornithological data on the summer and winter range of redstarts. Since the isolation of EgAn 1169-61 predates the Siberian isolates, Kemerovo virus is presumed to have been active in Siberia before its recorded date of isolation. Further, these data implicate migratory birds in the dispersal

of the virus over vast distances.

e. Ixodes persulcatus ticks vector the Siberian isolates and Ixodes ricinus the Czechoslovakian isolates. Geographic and microclimatic conditions now separate these two species. Thus, the two subtypes of virus appear to represent distinct populations evolved from a recent common ancestor. These experiments show that if the barriers to inter-subtypic reassortment were to be removed, the subtypes would reassort and interact genetically.

f. Inter- and intra-serocomplex hybridization and gene reassortment data on the other Kemerovo serogroup viruses are required to evaluate the taxonomic significance of the serocomplex designation relative to other Orbivrus serogroups.

This study was submitted for publication in Intervirology.

Table 3. Kemerovo Serocomplex Virus Isolates

Isolate	Isolation Source	Geographical Origin	Year[a]
Siberian Serotype			
EgAn 1169-61	*Phoenicurus phoenicurus*	Burg El Arab, U.A.R.	1961
KM 3	*Ixodes persulcatus*	Kutchum, U.S.S.R.	1962
L 75	human	Kutchum, U.S.S.R.	1962
R 9	*Ixodes persulcatus*	Romanovka, U.S.S.R.	1962
Kemerovo (R 10)	*Ixodes persulcatus*	Romanovka, U.S.S.R.	1962
R 54	*Ixodes persulcatus*	Romanovka, U.S.S.R.	1962
Czechoslovakian Serotype			
Kol 42	*Ixodes ricinus*	Koliba, Czechoslovakia	1963
Kol 152	*Ixodes ricinus*	Koliba, Czechoslovakia	1963
Kol 156	*Ixodes ricinus*	Koliba, Czechoslovakia	1963
Lip 10	*Ixodes ricinus*	Lipovnik, Czechoslovakia	1963
Lip 11	*Ixodes ricinus*	Lipovnik, Czechoslovakia	1963
Lip 53	*Ixodes ricinus*	Lipovnik, Czechoslovakia	1963
Lipovnik (Lip 91)	*Ixodes ricinus*	Lipovnik, Czechoslovakia	1963
Tribec	*Ixodes ricinus*	Tribec Mountains, Czechoslovakia	1963

[a]Represents the year in which the isolate was collected in the field.

Table 4. *In Vitro* Reassortment of Czechoslovakian and Siberian Isolates

Minimum Number of Genes Distinguishable[a]	MOI Ratio (PFU/cell) (P1:P2)	Observed Progeny (P1:P2: Reassortant)	Reassortant Genotype (Segment)[b] 1	2	3	4	5	6	7	8	9	10
			Cross: L 75 X EgAn 1169-61									
6	18:18	5:2:11	X	L 75	X	EgAn	L 75	X	L 75	L 75	L 75	X
			X	L 75	X	EgAn	L 75	X	EgAn	L 75	L 75	X
			EgAn	EgAn	X	EgAn	EgAn	X	EgAn	L 75	EgAn	L 75
			L 75	L 75	X	L 75	L 75	X	EgAn	L 75	L 75	EgAn
			X	EgAn	X	EgAn	EgAn	X	L 75	L 75	L 75	X
			X	EgAn	X	L 75	L 75	X	L 75	L 75	L 75	X
			X	EgAn	X	L 75	L 75	X	L 75	L 75	L 75	X
			EgAn	L 75	X	EgAn	EgAn	X	EgAn	L 75	EgAn	L 75
			L 75	EgAn	X	EgAn	L 75	X	EgAn	L 75	EgAn	L 75
			X	EgAn	X	EgAn	L 75	X	L 75	L 75	L 75	X
			X	EgAn	X	L 75	L 75	X	L 75	L 75	L 75	X
			Cross: Tribec (TRB) X Kol 152 (Kol)									
8	23:29	3:3:12	TRB	Kol	Kol	TRB	TRB	Kol	TRB	TRB	TRB	TRB
			Kol	Kol	Kol	Kol	Kol	Kol	Kol	Kol	TRB	Kol
			TRB	TRB	TRB	Kol	TRB	TRB	Kol	Kol	TRB	TRB
			TRB	TRB	TRB	TRB	TRB	TRB	TRB	TRB	Kol	TRB
			TRB	TRB	TRB	TRB	TRB	TRB	Kol	Kol	TRB	TRB
			TRB	TRB	TRB	TRB	TRB	TRB	TRB	TRB	Kol	TRB
			TRB	Kol	Kol	Kol	Kol	X	X	TRB	TRB	TRB
			TRB	Kol	Kol	Kol	Kol	X	X	Kol	TRB	TRB
			TRB	TRB	TRB	TRB	TRB	X	X	TRB	Kol	TRB
			TRB	TRB	Kol	TRB	TRB	X	X	TRB	TRB	TRB
			Kol	TRB	Kol	Kol	Kol	X	X	TRB	Kol	Kol
			TRB	TRB	TRB	TRB	TRB	X	X	TRB	Kol	TRB
			Kol	TRB	TRB	Kol	Kol	X	X	TRB	Kol	TRB
			Cross: L 75 X Tribec (TRB)									
7	65:20	21:9:2	L 75	L 75	L 75	TRB	X	L 75	L 75	X	L 75	X
			L 75	L 75	L 75	TRB	X	L 75	L 75	X	L 75	L 75
	65:40	13:41:6	L 75	L 75	L 75	L 75	TRB	L 75	L 75	L 75	L 75	L 75
			TRB	TRB	TRB	TRB	TRB	TRB	TRB	TRB	L 75	TRB
			L 75	L 75	L 75	TRB	L 75	L 75	L 75	L 75	L 75	L 75
			L 75	L 75	L 75	L 75	L 75	L 75	L 75	L 75	L 75	TRB
			TRB	TRB	TRB	TRB	TRB	TRB	TRB	TRB	L 75	TRB
			L 75	L 75	TRB	TRB	L 75	L 75	L 75	L 75	L 75	TRB

[a]The number of segments which have a significantly different mobility when the two parental viruses are electrophoresed in polyacrylamide. Thus, it reflects the number of genes for which the parental origin could be determined in progeny viruses. This represents a minimum number because resolution was often improved using end-labeled dsRNA.

[b]The parental origin of each segment is indicated by a single letter or number abbreviation, and segments of undetermined origin are indicated by the letter X.

II. IDENTIFICATION AND CLASSIFICATION OF ARBOVIRUSES

A. Study of viruses from Thailand and Nepal (R.E. Shope, R.B. Tesh, and B. Fonseca).

Thirteen viruses from Kampangphet, Thailand and five from Biratnagar, Nepal were received for identification from Dr. B. Innis, Armed Forces Research Institute for Medical Science, Bangkok. These were isolated in AP-61 mosquito cells and passaged at Yale in C6/36 cells.

Initial screening of 6 of the viruses by IFAT of infected C6/36 cells showed that BND85-14401 reacted with both alphavirus and flavivirus grouping antibody, indicating a mixed infection. Two others (Nep85-96 and B1726/85) reacted with the alphavirus grouping antibody alone. Two (BTK85-154 and BKP"u"CTT500 reacted with the flavivirus grouping antibody alone, and one (B1726/85) reacted with neither grouping reagents.

BND85-14401 was plaque-cloned and the progeny reacted only with the alphavirus fluid; mouse brain passage material reacted with tembusu by CF.

Two of the viruses from Nepal, NEP85-96 and NEP85-99, killed baby mice in 3-4 days. These were tested by CF with alphaviruses getah, bebaru, and chikungunya and were identified as very closely related to getah (Table 5). Neutralization tests will be done. This is the first identification of getah-related viruses from Nepal.

Five of the viruses killed baby mice by i.c. inoculation with average survival times of 7-9 days. These were confirmed as flaviviruses. Sucrose-acetone extracted mouse brain antigens were prepared. Three of them reacted by CF with Tembusu and to lower titers with Japanese encephalitis, Zika, and Sepik antibodies. Two others reacted in equal titers with JE and Tembusu antibodies (Table 6). Neutralization tests are planned for definitive identification.

Table 5

CF reactions of NEP85-96 and NEP85-99 antibodies with alphaviruses

	Mouse ascitic fluids			
Antigens	NEP85-96	NEP85-99	getah	chikungunya
NEP85-96	512/≥16*	512/≥16	1024/16	0/0
NEP85-99	1024/64	1024/≥256	256/8	0/0
getah	256/>256	64/1024	128/≥1024	not done
bebaru	8/4	not done	not done	not done
chikungunya	64/≥256	32/64	not done	≥1024/4

reciprocal of antibody/reciprocal of antigen titer; 0=<8/<4.

Table 6

CF reactions of 5 Thai flaviviruses with tembusu, Japanese encephalitis, Zika, and Sepik antibodies

	Mouse immune ascitic fluids			
Antigens	tembusu	Japanese encephalitis	Zika	Sepik
tembusu	512/≥512*	128/≥512	not tested	not tested
Japanese enc.	256/≥512	512/≥512	not tested	not tested
Zika	not tested	not tested	≥1024/32	0/0
Sepik	not tested	not tested	0/0	≥1024/128
KP84-425	512/≥512	512/128	32/8	256/8
KP84-326	256/≥512	256/128	32/8	128/8
KP84-308	512/≥512	16/32	0/0	0/0
BTK85154	256/≥512	64/32	0/0	0/0
BND85-14401	512/≥512	256/128	16/8	128/8

*reciprocal of antibody/reciprocal of antigen titer

B. Isolation of Sicilian sandfly fever virus from Egyptian phlebotomines (R.B. Tesh and A. El-Dessouky).

During October 1987, Mr. Ashrof El-Dessouky, a graduate student at Ain Shams University, Cairo, Egypt spent two weeks at YARU processing sandfly specimens which he had collected earlier in September, 1986 in Giza Governorate, Egypt. One of 40 pools processed yielded a virus, despite the less than optimal conditions under which the insects had been transported to the United States. This agent was shown by IFAT to be a member of the phlebotomus fever serogroup and by plaque reduction neutralization test to be indistinguishable from Sicilian sandfly fever virus. This isolation emphasizes once again that Sicilian virus (and Naples as well) is still endemic in many rural areas of the Middle East.

C. Isolation and characterization of new sandfly isolates from Colombia, Brazil and Ivory Coast (R.B. Tesh).

During 1987, an attempt was made to characterize eight probable new phlebovirus serotypes. The identificatin numbers of the viruses and their source and origin are as follows:

Identification number	Source	Geographic origin
CoAr 170396	Lutzomyia sp.	Colombia
BeAr 407981	Lutzomyia sp.	Brazil
Mariquita A	Lutzomyia sp.	Colombia
CoAr 171046	Lutzomyia sp.	Colombia
CoAr 171096	Lutzomyia sp.	Colombia
CoAr 170897	Lutzomyia sp.	Colombia
CoAr 171162	Lutzomyia sp.	Colombia
Odrenisrou	Culex albriventris	Ivory Coast

Each of these agents initially reacted by immunofluorescence with a phlebovirus grouping serum and with one or more specific phlebovirus immune mouse ascitic fluids. Each virus was then screened against a battery of 49 specific mouse immune ascitic fluids (MIAFs) by plaque reduction neutralization test. Although a few MIAFs are still being prepared, preliminary neutralization test results suggest that each of these agents represents a new phlebotomus fever virus subtype (Table 7).

D. Identification of MSP-18 virus, a Cache Valley subtype from Panama (R.E. Shope and L.T. Figueiredo).

MSP-18 virus was isolated from the acute phase blood of a United States military recruit on maneuvers in Panama. The virus was referred by J. Mangiafico, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick. MSP-18 was identified as a member of the Bunyamwera serogroup by CF; it reacted with the Bunyamwera grouping mouse ascitic fluid. CF tests with members of the Bunyamwera serogroup showed it to be in the Bunyamwera complex, indistinguishable by CF from Cache Valley, Bunyamwera, Maguari, Playas and Xingu (Table 8). By neutralization tests in 96-well plates using infected Vero cells and ELISA as indicator, MSP-18 was closely related to Cache Valley virus. Additional tests at Fort Detrick confirmed that MSP-18 virus is a subtype of Cache Valley virus.

Table 7. Results of plaque reduction neutralization with 8 probable new phleboviruses

Antiserum	VIRUS							
	CoAr 170396	BeAr 407981	Mariquita A	CoAr 171046	CoAr 171096	CA Ar 170997	CoAr 171162	Odrenisrou
Aquacate	0	0	0	0	0	0	0	0
Alenquer	0	0	0	0	0	0	0	0
Anhanga	0	0	0	0	0	0	0	0
Arbia	0	0	0	0	0	0	0	1:20
Arumowot	0	0	0	0	0	0	0	0
Belterra	0	0	0	0	0	0	0	0
Buenaventura	0	0	0	0	0	0	0	0
Bujaru	0	0	0	0	0	0	0	0
Cacao	0	0	0	0	0	0	0	0
Caimito	0	0	0	0	0	0	0	0
Candiru	0	0	0	0	0	0	0	0
Chagres	0	0	0	0	0	0	0	0
Chilibre	0	0	0	0	0	0	0	0
Corfu	0	0	0	0	0	0	0	0
Frijoles	0	0	0	0	0	0	0	0
Gabek Forest	0	0	0	0	0	0	0	0
Gordil	0	0	0	0	0	0	0	0
Icoaraci	0	0	0	0	0	0	0	0
Itaituba	0	0	0	0	0	0	0	0
Itaporanga	0	0	0	0	0	0	0	0
Joa	0	0	0	0	0	0	0	0
Karimabad	0	0	0	0	0	0	0	0
Munguba	0	0	0	0	0	0	0	0
Naples	0	0	0	0	0	0	0	0
Nique	0	0	0	0	0	0	0	0
Oriximina	0	0	0	0	0	0	0	0
Pacui	0	0	0	0	0	0	0	0
Punta Toro	0	0	0	0	0	0	0	0
Rift Valley Fever	0	0	0	0	0	0	0	0

(continued next page)

Table 7. Results of plaque reduction neutralization with 3 probable new phleboviruses (continued)

Antiserum	CoAr 170396	BeAr 407981	Mariquita A	CoAr 171046	CoAr 171096	CA Ar 170897	CoAr 171162	Odrenisrou
				VIRUS				
Rio Grande	0	0	0	0	0	0	0	0
Saint Floris	0	0	0	0	0	0	0	0
Salehabad	0	0	0	0	0	0	0	0
Sicilian	0	0	0	0	0	0	0	0
Tehran	0	0	0	0	0	0	0	0
Toscana	0	0	NT	0	0	0	0	0
Turuna	0	0	0	0	0	0	0	0
Urucuri	0	0	0	0	0	0	0	0
BeAr 413570	0	0	NT	0	0	0	0	0
CoAr 170152	0	0	0	0	0	0	0	0
CoAr 170255	0	0	0	0	0	0	0	0
Mariquita A	0	0	1:320	0	0	0	0	0
Nique 1-Q	0	0	0	0	0	0	0	0
BeAr 407981	0	1:160	0	0	0	0	0	0
CoAr 170396	NT			0	0	0	0	0
CoAr 171046				NT	NT	NT	0	NT
CoAr 171096	10		0	0	1:40	0	0	0
CoAr 171162	0		0	0	0	0	1:40	0
CA Ar170897	0		0	0	0	1:320	0	0
Odrenisrou	NT	NT	NT	NT	NT	NT	NT	NT

NT = not tested

Table 8

Complement fixation test reactions of MSP-18 virus

with viruses in the Bunyamwera serogroup

	Antibody									
Antigen	MSP18	CV	MAG	BUN	PLA	XIN	KAI	TAI	GUA	GER
MSP18	512/32*	512/32	32/32	128/32	512/32	128/32	0/0	8/4	0/0	0/0
Cache Valley	512/256	512/256								
Maguari	512/64		32/64							
Bunyamwera	512/64			512/256						
Playas	512/8				512/8					
Xingu	128/8					512/8				
Kairi	8/64						128/256			
Tataguine	8/256							128/256		
Guaroa	32/256								128/256	
Germiston	8/8									32/8

*reciprocal of antibody titer/reciprocal of antigen titer; 0/0 = <8/<4.

III. CHARACTERIZATION OF MONOCLONAL ANTIBODIES

A. Preparation of monoclonal antibodies against VSV-Indiana complex viruses (W.R. Chen and R.B. Tesh).

Vesicular stomatitis is a viral disease of bovines, equines and swine, which clinically is indistinguishable from foot-and-mouth disease. Four viruses in the vesicular stomatitis virus (VSV) serogroup have been associated with epizootics of vesicular disease among large domestic animals in the New World. Three of these agents, (VSV-Indiana, VSV-Alagoas and Cocal) are closely related antigenically and comprise the VSV-Indiana complex. In fact, veterinary health personnel often refer to these three agents as VSV-Indiana types 1, 2, and 3, respectively.

By ELISA and indirect fluorescent antibody test (IFAT), these three viruses are closely related and are difficult to differentiate. Table shows results of a standard IFAT, using Indiana, Alagoas and Cocal antigens (infected Vero cells) and polyclonal mouse immune ascitic fluids prepared against the three viruses. By this technique, the three agents are not easy to differentiate.

Accordingly, a library of monoclonal antibodies was generated against each of the three viruses. The resulting hybridomas were screened for serotype-specific activity by IFAT and by ELISA. Table illustrates results obtained in IFAT with five selected monoclonal antibodies. Monoclonal ALG-1-1-G2-F3 reacted with all three antigens, while the remaining four antibodies were serotype specific.

During the past year, Dr. Peter Fernandez, a veterinarian and also public health student, took selected VSV-Indiana complex monoclonal antibodies to the Pan American Health Organization Foot-and-Mouth Disease Diagnostic Laboratory in Rio de Janeiro to test them against field isolates of VSV from South America. This work is still in progress. Potentially these monoclonal abtibodies might facilitate the identification of vesicular stomatitis viruses and their differentiation from foot-and-mouth disease viruses.

Preliminary characterization of the monoclonal antibodies indicates that most are made to non-structural proteins.

Table 9

Results of IFAT with VSV-Indiana, VSV-Alagoas and Cocal viruses using polyclonal mouse immune ascitic fluids (MIAF)

MIAF	Indiana	Alagoas	Cocal
Indiana	320*	320	320
Alagoas	640	≥2,560	640
Cocal	320	160	2,560

*Reciprocal of highest positive MIAF dilution

Table 10

Results of IFAT with VSV-Indiana, VSV-Alagoas and Cocal viruses using selected monoclonal antibodies

Monoclonal antibody	Antigen		
	Indiana	Alagoas	Cocal
ALG-1-1-G2-F3	+	+	+
IND-1-4-G3-B4	+	0	0
IND-1-2-G12-E10	+	0	0
ALG-1-2-E6-5A	0	+	0
COC-2-3-F1-G10	0	0	+

B. Preparation of monoclonal antibodies against Cache Valley virus (R.E. Shope and G.H. Tignor).

BALB/c mice were immunized with the Texas 7856 strain of Cache Valley virus and spleens were fused with Ag8 myeloma cells. The resulting hybridomas were selected for their ability to react in ELISA with sucrose-acetone extracted mouse brain antigen of Cache Valley virus. One of the hybridomas has been cloned and ascitic fluid of mice reacts well with the antigen. It is intended to use this antibody as coating of plastic plates for capturing Cache Valley antigen.

C. A conserved epitopic region on alphavirus nucleocapsid protein (I. Greiser-Wilke, V. Moennig, O. Kaaden, L.T.M. Figueiredo, and R.E. Shope).

In a collaborative study with the Institute for Virology, Hannover Veterinary School, Hannover, Germany, monoclonal antibodies to Semliki Forest virus (SFV) were tested with a battery of alphaviruses. Monoclonal antibodies were made in Germany using P3-NS1/AG-4-1 myeloma cells and selected by ELISA with Sindbis antigen. The antibodies belonged to the IgG1 (C23), IgG2a (C27, C42), IgG2b (C2, C12, C16, C28, C29, C30, C56, C63) and IgG3 (C18, C22, C36) subclasses. Under non-reducing conditions, one antibody reacted with E1, 6 with E2, and 14 with the NC protein in immunoblot ELISA. The antibodies were concentrated from serum-free cell culture supernatant fluids and purified by protein A-Sepharose column chromatography, then conjugated to peroxidase.

Cross reactivity of the monoclonal antibodies was determined in two different tests with 27 alphaviruses (Table 11). Two types of ELISA were done, antigen capture and an indirect ELISA in which infected C6/36 cells were grown in 96-well plates and fixed with 3.3% formalin. For the antigen capture test stock viruses were prepared from virus-infected C6/36 or BHK-21 cell culture supernatant fluids. The antigens were inactivated with 0.3% beta-propiolactone. Both tests, antigen capture and ELISA, yielded consistent results.

The results of the antigen capture tests are summarized in Table 11. None of the antibodies reacted with Barmah Forest (BF) or with viruses of the VEE antigenic complex (VEE, Bijou Bridge, Everglades, Mucambo, and Pixuna). Because of differences in their reactivity with Fort Morgan (FM), Y62-33, Whataroa (WHAT), and chikungunya (CHIK) viruses, the antibodies were divided into six types. The first type reacted with the four viruses (C12, C27, and C56). Antibodies of the second type reacted with FM, WHAT and Y62-33, but not with CHIK virus (C2, C18, C22, and C23). Type three antibodies (C28 and C63) did not react with WHAT and CHIK, while type 4 (C42) did not react with WHAT and Y62-33 viruses. Type five antibody (C16) did not react with FM or with CHIK viruses. Antibodies of type six (C29, C30, and C36) reacted only with WHAT virus. From these results we concluded that the antibodies recognized six different epitopes on the NC-protein. Reciprocal antibody blocking tests using Sindbis virus showed that all antibodies competed with each other. The blocking of antibody pairs was always symmetrical, and no one way reactions were observed. The binding of the NC protein specific antibodies was not influenced by the presence of any of the two E2 glycoprotein specific antibodies C3 and C8, and vice versa. Therefore all epitopes were either identical within one reactivity type or clustered on a single antigenic region of the protein.

Table11: Reactivity of monoclonal antibodies against Semliki Forest virus as determined by antigen capture enzyme-linked immunosorbent assay

VIRUS AND SUBTYPE*1	STRAIN	REACTION OF MONOCLONAL ANTIBODIES type*2 I	II	III	IV	V	VI
EASTERN ENCEPHALITIS	Ten Broeck	+	+	+	+	+	+
VENEZUELAN ENCEPHALITIS							
V.encephalitis	Trinidad donkey	-	-	-	-	-	-
V.encephalitis	TC83	-	-	-	-	-	-
Everglades	Fe 3 7C	-	-	-	-	-	-
Mucambo	BeAn 8	-	-	-	-	-	-
Pixuna	BeAr 35645	-	-	-	-	-	-
Bijou Bridge	CM4-146	-	-	-	-	-	-
WESTERN ENCEPHALITIS							
W. encephalitis	McMillan	+	-	+	-	+	-
W. encephalitis	Y62-33	+	+	+	-	+	-
Fort Morgan	CM4-146	+	+	+	+	-	-
Whataroa	M78	+	+	-	-	-	-
Sindbis	AR86	+	+	+	+	+	-
Sindbis	AR339	+	+	+	+	+	+
-Kyzylagach	LEIV65A	+	+	+	+	+	+
SEMLIKI FOREST							
Semliki Forest	Simons	+	+	+	+	+	+
Semliki Forest	R 16225	+	+	+	+	+	+
Chikungunya	Ross	+	-	-	+	-	-
-O'Nyong Nyong	Gulu	+	+	+	+	+	+
Getah	R 19222	+	+	+	+	+	+
-Sagiyama	31407 Harv.	+	+	-	+	+	+
-Bebaru	MM 2354	+	+	+	+	+	-
-Ross River	T 48	+	+	+	-	+	+
Mayaro	Tr 15537	+	+	+	+	+	+
-Una	BeAr 13136	+	+	+	+	+	-
NDUMU	SA AR 211	+	+	+	+	+	+
MIDDELBURG	SA AR 749	+	+	+	+	+	-
BARMAH FOREST	Aus. BH-2193	-	-	-	-	-	-

1* From Shope (1985) and Calisher et al.(1988)
2* Type I (C12, C27, C56); type II (C2, C18, C22, C23) type III (C28, C63); type IV (C42); type V (C16), and type VI (C29, C30, C36)

IV. OUTBREAK INVESTIGATION

A. Evidence that Cache Valley virus induced arthrogryposis and hydranencephaly in sheep in Texas and Nebraska (R.E. Shope and R. Yedloutschnig)

Dr. Gary Combs of the U.S.D.A. Animal and Plant Health Inspection Service (APHIS) requested that YARU assist in the diagnosis of an outbreak of arthrogryposis and hydranencephaly (AGH) occurring during January 1987 at San Angelo State University and Texas A&M, Texas, and Clay Center, Nebraska. These were experimental flocks in which rams from New Zealand had been imported. Drs G. Svetlik and L. Williams of APHIS collected sera to investigate the possibility that the outbreak was caused by Akabane virus, implicated in AGH outbreaks in Australia, Japan, and Israel. Studies at the APHIS Laboraatory at Plum Island, NY demonstrated low levels of neutralizing antibody to Akabane virus in one of the rams, but not in most of the ewes that had lambs affected by the disease. Bluetongue, which can cause AGH, had also been ruled out by agar gel diffusion tests.

Sera from ewes were tested by ELISA for the Simbu group viruses, Buttonwillow and Mermet, on the hypothesis that perhaps viruses related to Akabane were implicated. Since no known positive sheep sera for Buttonwillow and Mermet were available, Cache Valley antigen (known to be enzootic in Texas) was included in order to assure that there would be control positive reactions in the ELISA.

ELISAs with Buttonwillow and Mermet antigens were negative. The results with Cache Valley antigen are shown in Table 12. Twenty-eight of 29 ewes with affected lambs from Texas A&M and San Angelo State University, TX, were positive to Cache Valley antigen. Nearly all of the ewes with affected lambs from Nebraska were also positive. Ewes that gave birth to normal lambs were negative in Texas and had lower prevalence of antibody in Nebraska, although the number of negative control ewes was small. Neutralization test results at U.S.D.A., Plum Island, and at the Texas A&M University (Shan-Ing Chung and Ellen Collisson) correlated with the ELISA results. The Texas group demonstrated Cache Valley neutralizing antibody in sera of pre-colostral lambs with AGH indicating they were likely infected in utero.

Table 12

Cache Valley ELISA results with sera of ewes from Texas and Nebraska

	Ewes positive	Number tested	Location
AGH and aborted	4	4	San Angelo State
Normal lambs	0	2	San Angelo State
AGH and aborted	24	25	Texas A&M
AGH and aborted	12	14	Clay Center, Nebraska
Normal lambs	3	6	Clay Center, Nebraska

Of 360 lambs born between December 1986 and February 1987 at San Angelo, TX, 19.2% had AGH. The epizootic peaked at the end of January 1987. Attempts are under way at Texas A&M University to reproduce the disease in pregnant sheep by intrauterine inoculation of Cache Valley virus.

V. RETROVIRUS REFERENCE STUDIES

A. Attempt to characterize a novel virus from persons with AIDS (R.E. Shope, R. Cedeno and G.H. Tignor).

Dr. Shyh-Ching Lo of the Armed Forces Institute of Pathology, Washington, DC provided spot slides for IFA tests of a new viral agent. The virus was isolated from patients with Kaposi's sarcoma by transfection of genetic material into NIH/3T3 mouse cells. According to Dr. Lo the virus was 140-280 nm in diameter, nearly spherical, and had a well-defined outer limiting membrane and tightly packed internal nucleocapsid (Lo, S.-C. Isolation and identification of a novel virus from patients with AIDS, Am. J. Trop. Med & Hyg 35:675-676, 1986).

The spot slides were tested and found negative with 31 grouping ascitic fluids representing alphaviruses, flaviviruses, bunyaviruses, rhabdoviruses, arenaviruses, vaccinia, herpes simplex, NDV, mouse hepatitis, and several unclassified arboviruses and rodent viruses.

B. Establishment of collaborative HIV diagnostic capability in Bogota, Colombia (R.B. Tesh and J. Boshell).

In order to establish field capability in HIV diagnosis, normal and infected H-9 cells were transported during 1987 to the Instituto Nacional de Salud. YARU will receive HIV positive cultures for confirmation of identity of HIV isolates, and will store infected cells in liquid nitrogen as part of the low-passage virus collection (see section VII).

VI. DEVELOPMENT OF NEW TECHNIQUES

A. Exploration of JE- trpE Fusion Proteins as analytical, vaccine, and diagnostic reagents (P.W. Mason)

The flavivirus envelope glycoprotein (E) contains antigenic determinants that are important for virus uptake and pathology. We have undertaken the expression of Japanese encephalitis virus (JE) cDNA in E. coli as a method for: 1) localizing important antigenic determinants within the E protein amino acid sequence, 2) the production of recombinant vaccine, and 3) production of serological diagnostic reagents. This work was initiated at the University of Massachusetts where Dr. P. Mason received postdoctoral training with Dr. M. J. Fournier and Dr. T. L. Mason. The work has continued since Dr. P. Mason's arrival at YARU and represents an ongoing collaboration with the workers at the University of Massachusetts. In addition, the mouse protection experiments referred to in this report were performed in collaboration with Dr. Charles Hoke and coworkers at the Walter Reed Army Institute of Research.

Preliminary E. coli expression of JE cDNA using the bacteriophage lambda gtll expression system (1,2) identified two separate regions of the E protein that were efficiently expressed as JE-beta-galactosidase fusion proteins and reacted with antibodies present in polyclonal hyperimmune mouse ascitic fluid (HMAF). The fusion protein derived from one of these regions which we define as Domain II, is encoded within amino acid residues 280 to 414 of the E protein. Domain II contains the epitopes recognized by 10 monoclonal antibodies (MAbs) generated from JE-infected mice. These 10 MAbs appear to recognize different epitopes on the E protein based on competitive binding analyses (Dr. D. S. Burke, WRAIR, personal communication), cross-reactivity with other flaviviruses, and neutralization titers.

One goal of the experiments described here was to map the epitopes of the available MAbs relative to the predicted amino acid sequence of the JE E protein. Our strategy utilized exonuclease digestion of viral cDNA fragments to construct a series of bacterial expression plasmids that expressed different portions of the E protein. To this end, the cDNA fragment present in the shortest immunoreactive lambda gtll clone was subcloned into an easily manipulatable plasmid expression vector (pATH; 3) yielding a JE-pATH recombinant plasmid (pATH#6). E. coli cells harboring this plasmid could be induced to produce large quantities of a fusion protein containing portions of the trpE gene product and the JE E protein. Progressive Bal31 exonuclease digestion of the expressed viral sequences, followed by recloning in the pATH plasmid, was used to generate a series of fusion proteins that could be used to map the epitopes recognized by the MAbs and HMAF. Figure 1 shows the amino acid residues expressed by a nested set of pATHC recombinants generated by this technique.

Analyses of the immunological properties of the domain II fusion proteins specified by these plasmids have been used to map the epitopes recognized by HMAF and all 10 MAbs (including four MAbs that show strong neutralizing titers) to a region between amino acid 303 and 396 of the E protein. These results are clearly demonstrated by the ELISA data shown in Table 13. The large size of this region (about 20% of the 55 kD E protein),

and the inability to dissect this region using our deletion strategy, suggested that domain II consists of a large tertiary protein structure. The presence of a Cys residue at the N-terminal limit of the smallest immunoreactive recombinant protein prompted biochemical studies on the authentic viral protein. The data shown in Table 14 clearly demonstrate the importance of a Cys-Cys bond in the presentation of the epitope recognized by these MAbs. Biochemical studies on the E protein of the closely related West Nile virus (4) have shown that two cys residues, equivalent to those found at positions 304 and 335 in the JE E protein (2), are disulfide bonded to each other. These data are consistent with the importance of this disulfide bond in the presentation of this epitope in infected animals. As expected from the ELISA results shown in Table 13, reduction and alkylation of the pATH#6 fusion protein indicated that this disulfide bond was present in the recombinant fusion protein (results not shown).

Initial experiments to evaluate the ability of domain II-trpE fusion proteins to act as protective immunogens were met with dissappointing results. Although animals immunized by intraperitoneal injection with electrophoretically purified fusion protein specified by the pATH#6 recombinant (in Freunds adjuvant, followed by two boosts) produced antibodies that reacted with the viral protein, none of the animals immunized with these proteins produced neutralizing antibody titers. In addition, similar immunizations with the same fusion protein, performed by Dr. Charles Hoke and coworkers at WRAIR, failed to protect mice from lethal challenge from JE. Furthermore, the ascites fluids obtained from mice that were immunized with this fusion protein bound to unreduced, reduced, and reduced & alkylated proteins (Table 15), suggesting that the immunological response to the fusion protein immunogens was amino acid-sequence specific and did not recognize the important neutralizing-conformational epitope found on the native viral protein.

These fusion protein reagents, which are extremely cheap and easy to produce have been investigated as potential reagents for typing antiviral antibodies. Initial experiments showed that the fusion proteins readily distinguised antibodies produced to different flaviviruses. Table 16 shows the specific reactivities of the fusion protein produced by the pATH recombinant pHB3-3 as targets in ELISA assays of murine ascites fluids raised to several different flaviviruses.

References:

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3. Dieckmann, C.L., and Tzagoloff, A. 1985. Assembly of the mitochondrial membrane system. CBP6, a yeast nuclear gene necessary for synthesis of cytochrome b. J. Biol. Chem. 260:1513-1520.

4. Nowsk, T., and Wengler, G. 1987. Analysis of disulfides present in the membrane proteins of the West Nile flavivirus. Virol. 156:127-137.

Figure 1

JE amino acid sequences expressed by JE domain II pATH recombinants. (Amino acid residues 313-388, shared by all recombinants, are deleted from figure to conserve space).

C-TERMINAL DELETIONS

```
                                                                    a
      280        290        300        310        390        400        410
       *          *          *          *          *          *          *
pATH#6 LTSGHLKCRLKMDKLALKGTTYGMCTKEFSFAK /  / DKQINHHWHKAGSTLGKAFSTTLKGA
pKS40  LTSGHLKCRLKMDKLALKGTTYGMCTEKFSFAK /  / DKQINHHWHKAG
pKS29  LTSGHLKCRLKMDKLALKGTTYGMCTEKFSFAK /  / DKQINHHWHK
pKS30  LTSGHLKCRLKMDKLALKGTTYGMCTEKFSFAK /  / DKQINHHW
pKS26  LTSGHLKCRLKMDKLALKGTTYGMCTEKFSFAK /  / DKQIN
pKS19  LTSGHLKCRLKMDKLALKGTTYGMCTEKFSFAK /  / D
```

N-TERMINAL DELETIONS

```
      280        290        300        310        390        400
       *          *          *          *          *          *
pKS40 LTSGHLKCRLKMDKLALKGTTYGMCTEKFSFAK /  / DKQINHHWHKAG
       HB2-2  RLKMDKLALKGTTYGMCTEKFSFAK /  / DKQINHHWHKAG
              HB2-10 LKGTTYGMCTEKFSFAK /  / DKQINHHWHKAG
                     HB3-3  MCTEKFSFAK /  / DKQINHHWHKAG
                            J-15  FAK /  / DKQINHHWHKAG
```

Table 13. ELISA results show that the antigenic determinants recognized by all 10 MAbs and HMAF react with the same large region of domain II of the JE E protein, and that there are no fusion proteins that react with a subset of the MAbs.

	MOUSE ASCITES FLUID											
trpE CLONE[a]	HMAF	NAF	J2-5A11	J2-5F1	J2-7F12	J3-10E1	J3-11B9	J3-11G5	J3-11H7	J3-12H11	J3-14E6	J3-14H5
pATH#6	1.2	-[b]	>1.7	1.4	0.10	0.26	>1.7	1.4	>1.7	>1.7	1.4	1.7
pKS40	1.4	-	1.5	1.2	0.07	0.22	>1.7	1.5	>1.7	>1.7	1.6	>1.7
pKS29	0.70	-	1.1	0.75	0.04	0.16	>1.7	1.0	>1.7	>1.7	1.1	1.5
pKS30	0.44	-	0.73	0.41	0.04	0.16	1.4	0.71	1.4	1.5	0.85	0.58
pKS26	-	-	0.05	-	-	-	-	0.09	-	0.22	-	-
pKS19	-	-	0.06	-	-	-	-	0.11	-	-	0.25	-
HB2-2	1.5	-	>1.7	1.7	0.10	0.26	>1.7	1.7	>1.7	>1.7	>1.7	>1.7
HB2-10	1.4	-	>1.7	1.7	0.15	0.31	>1.7	1.7	>1.7	>1.7	1.7	1.7
HB3-3	1.4	-	1.7	1.7	0.19	0.36	>1.7	1.7	>1.7	>1.7	>1.7	1.7
J15	0.02	-	-	-	-	-	-	0.12	0.02	-	-	-

[a] Insoluble fractions isolated from the indicated clones were prepared by standard techniques, and the material derived from approxiamately 20 microliters of induced bacterial culture was used to coat each well.
[b] (-) designates O.D. values less than or equal to 0.01.

Table 14. ELISA reactivity of reduced, reduced and alkylated or alkylated preparations of the E protein.

	E PROTEIN ANTIGEN[a]			
ASCITES FLUID	UNTREATED	REDUCED	REDUCED & ALKYLATED	ALKYLATED
JE HMAF	>1.75	0.68	0.53	1.56
NAF	0.03	-[b]	-	0.02
J2-5A11[c]	>1.75	0.45	-	1.4

[a] PEG precipitate of JE-infected Vero cell supernatants, dissolved in 0.1% SDS was used as the source of E protein. Samples were reduced by treatment with 2 mM dithiothreitol for 30 min. at 60°C and alkylated by treatment with 8 mM iodoacetamide for 30 min. at 60°C.
[b] (-) designates O.D. values less than or equal to 0.01.
[c] All MAbs showed a similar profile of reactivity (results not shown).

Table 15. ELISA reactivity of ascitic fluids from four balb/c mice immunized with pATH#6 derived fusion proteins with reduced, reduced and alkylated or alkylated preparations of the E protein.

ASCITES FLUID	VIRION ANTIGEN[a] UNTREATED	REDUCED	REDUCED & ALKYLATED	ALKYLATED
F1	1.30	1.55	1.26	1.13
F2	0.21	0.27	0.19	0.19
F3	0.14	0.25	0.13	0.15
F4	0.03	0.06	0.01	0.02

[a] Antigen was prepared as described in Table .

Table 16. Specific[a] ELISA reactivity of flavivirus MIAFs with the domain II fusion protein produced by pHB3-3.

MIAF \ DIL.	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800
JE	1.49	1.54	1.51	1.23	1.07	0.72	0.33	0.26
KUN	0.34	0.18	0.08	0.04	0.02	-[b]	-	-
MVE	0.37	0.22	0.15	0.07	0.04	0.02	-	-
WN	0.37	0.25	0.18	0.09	0.05	0.03	0.02	-
SLE	-	-	-	-	-	-	-	-
DEN1	-	-	-	-	-	-	-	-
DEN2	-	-	-	-	-	-	-	-
DEN3	-	-	-	-	-	-	-	-
DEN4	-	-	-	-	-	-	-	-
YF	-	-	-	-	-	-	-	-

[a] O.D. values are the difference between the reactivity with the insoluble fraction derived from pHB3-3 fusion protein and the average reactivity with the pJ15 and pKS26 fusion proteins. Reactivities with the pJ15 and pKS26 fusion proteins ranged between 0.27 and 0.58 (for the 1:100 dilution) with the majority of values around 0.3.

[b] (-) designates O.D. values less than or equal to 0.01.

B. Molecular epidemiology of dengue viruses (R. Rico-Hesse: project supported in part by The Rockefeller Foundation)

Studies are under way to determine the evolutionary pathways of dengue viruses and, in this manner, determine the epidemiologic relationships of strains isolated throughout the world. Methods for determining genetic, and thus evolutionary, relationships of large numbers of viruses include serology, oligonucleotide fingerprinting, restriction enzyme mapping, cross-hybridization, and primer extension sequencing. The first method measures homologies of amino acid sequences encoded by nucleic acids, while the remainder use nucleic acid sequences to distinguish among viruses. Primer extension sequencing is the most valuable of these genomic methods since it yields information that is reliable and easy to interpret and compare among strains. It has the advantage that concise, selected regions of the genome can be compared. Viruses classified within a serotype can be compared and categorized evolutionarily based on sequence differences, and relationships graphically represented as a tree. The geographic origin of a virus can be deduced from the evolutionary tree, since viruses studied so far tend to evolve naturally in geographic clusters or econiches, the size of which varies according to transmission patterns. Dengue virus, like other RNA viruses, is amenable to this approach because of its high rate of mutation at the nucleotide level.

Preliminary results suggest that dengue 1 isolates can be classified geographically by comparison of nucleotide sequences obtained from the NS1 region of the genome. Mutations in this region occur mainly in the third position of the codon, that is, most of the mutations are silent, and an average mutation rate of 7% is seen when comparing 18+ strains of serotype 1. This is approximately one half the rate at which polioviruses mutate and this difference may be due to several factors which are unknown as of yet. Table 17 lists the isolates of dengue 1 analyzed to date. Isolates from Southeast Asia are distinct from those from the Americas and Africa, and some isolations made within the same country, at different times, also differ. The analysis of many more isolates is necessary to determine the specific region of the NS1 gene which will yield most epidemiologic information, and to select areas suited for the design of specific hybridization probes, which can be used for monitoring virus transmission.

Table 17

List of dengue-1 isolates used in molecular epidemiology studies

strain	passage	source	locality	year
Hawaii	monkey 1, mosq.6	human	Hawaii	1945
228682	mosq. ?, C6/36 1	human	Manila	1974
30130	mosq. 4	human	Fiji	1975
222683	mosq. ?, C6/36 1	human	Fiji	1975
228686	mosq. ?, C6/36 1	human	Burma	1976
16299	mosq. ?, C6/36 1	human	Nauru Islands	1977
228690	mosq. ?, C6/36 1	human	Jamaica	1977
1186	mosq. 2, C6/36 1	human	Jakarta	1977
1236	mosq. 2, C6/36 1	human	Jakarta	1978
29117	mosq. 2	human	Bandia, Senegal	1979
PUO359	C6/36 1	human	Thailand	1980
1298	mosq. 2, C6/36 1	human	Mexico	1980
1335	mosq. 2, C6/36 1	human	Colombo, Sri Lanka	1981
1344	mosq. 2, C6/36 1	human	Mexico	1982
1351	mosq. 2, C6/36 1	human	Colombia	1982
1412	mosq. 2, C6/36 1	human	Mexico	1983
1378	mosq. 2, C6/36 1	human	Mexico	1983
1413	mosq. 2, C6/36 1	human	Haiti	1983
347869	C6/36 3	human	Colombia	1985
CEA126		human	Ceara, Brazil	1987

VII. LOW PASSAGE VIRUS COLLECTION (R.B. Tesh and R.E. Shope)

In response to suggestions of the American Committee on Arboviruses' Subcommittee for the Collection of Low Passage Arbovirus Strains (SCLAS), we initiated a collection of such strains. The purpose was to collect original or low passage strains of arboviruses of medical or veterinary importance from representative geographic regions of the world and from different time periods. Such strains are often difficult to obtain and are very useful in studies of arboviral genetics and pathogenesis. Arbovirus field laboratories throughout the world were requested to submit original or low passage material for this collection, and many generously responded.

Virus specimens submitted were subsequently passaged in C6/36 or Vero cells. This material was used to prepare virus stocks which were lyophilized and stored. Because of the large number of virus specimens received, the identification of each strain has not yet been confirmed. The identity listed for each strain is that attributed by the donor.

A complete listing of the arbovirus strains now lyophylized is given in Table 18. Ampoules of any of these agents are available on request.

Abbreviations shown under passage history are as follows: suckling mouse (SM), Vero cells (Vero), C6/36 clone of Aedes albopictus cells (C6/36), Aedes pseudoscutellaris cells (AP-61), primary chick embryo (PCE), inoculation of live mosquitoes (MOSQ). The number following the abbreviation refers to the frequency of passage. For example, SM #1, Vero #1 would mean one passage each in suckling mice and in Vero cells.

VIII. ARBOVIRUS BULLETIN BOARD, REFERENCE, AND DATA ACCESS (D.L. Knudson and P.W. Mason)

A phone-accessible computer network is available by calling 203/785-2912. Prospective users may request a password at the time of initial contact. Available are a) a catalogue of available orbiviruses, b) sequence data for BT 17, Sindbis, VSV Indiana, WN, and YF 17D, and c) selected arbovirus references. Arbovirologists and other interested persons are encouraged to use this service. In the next year it is planned to add flavivirus sequences (under sponsorship of WHO) and to begin listing reagents available for distribution from YARU.

IX. DISTRIBUTION OF REAGENTS, WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH (R.E. Shope, R.B. Tesh, and G.H. Tignor)

A total of 385 ampoules of reagents including virus stocks (122), antigens (77), and antibodies (186) were distributed to laboratories in 12 countries plus 8 states in the USA. These included reagents to 70 different serotypes of arboviruses. In addition, 4 shipments of arthropods and 13 mosquito cell lines were also distributed.

Table 18. SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
Alajuelva	76V-2441	Mosq #1,C6/31#1	Aedeomyia squamipennis	Vinces, Ecaudor	May 1975
Arboledas	CoAr 171000	Vero #1	Lutzomyia spp.	Arboledas,N.S.,Colombia	1986
Arboledas	CoAr 170150	Vero #2	Lutzomyia spp.	Arboledas,N.S.,Colombia	1984
Cache Valley	69V-2152	C6/36 #1	Culiseta inornata	Umatilla Co., Oregon	1969
Cache Valley	RU-68	Vero #1	Aedes sollicitans	Dennisville,New Jersey	Sep 1982
Calif.enceph	BFN-2130	C6/36 #1	Aedes melanimon	Butte Co., California	May 1970
Calif.enceph.	BFN-3931	C6/36 #1	Aedes melanimon	Butte Co., California	Aug 1971
Calif.enceph.	E-19932	C6/36 #1	Aedes melanimon	Kern Co., California	Sep 1981
Calif.enceph.	Kern 175-82	C6/36 #1	Aedes melanimon	Kern Co., California	Sep 1982
Chagres	PaAr3419	Vero #2	Lu. sanguinaria	Bayano, Panama	Oct 1976
CHIK	RSU1	Vero #2	human serum	Ambon Island, Indonesia	1985
CHIK	37941	C6/36 #2,Vero #1	Aedes furcifer	Kedougou, Senegal	198?
CHIK	37937	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou, Senegal	1983
CHIK	37950	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou, Senegal	1983
CHIK	37997	AP-61 #1, C6/36 #1	Ae. furcifer	Kedougou, Senegal	1983
CHIK	37953	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou, Senegal	1983
CHIK	37963	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou, Senegal	1983
CHIK	1455/75	Mosq #2,C6/36 #1	human serum	Bangkok, Thailand	1975
CHIK	PO-731460	Vero #1, Mosq #1	human serum	Barsi, India	1973
DEN-1	INS-347869	C6/36 #3	human serum	Caqueta, Colombia	1985
DEN-1	Fiji 40170	Mosq?,C6/36 #1	human serum	Fiji	1975
DEN-1	Manila 19076	Mosq?,C6/36 #1	human serum	Manila, Philippines	1974
DEN-1	Burma 10378	Mosq?,C6/36 #1	human serum	Burma	1976
DEN-1	Jamaica 44684	Mosq.?, C6/36 #1	human serum	Jamaica	1977
DEN-1	PUO-359	C6/36 #1	human serum	Bangkok, Thailand	1980

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
DEN-1	Nauru 16299	Mosq. C6/36 #1	human serum	Nauru	1974
DEN-1	Dak H29177	Mosq. #1,C6/36 #1	" "	Bandia, Senegal	1979
DEN-1	1186	Mosq. #2,C6/36 #1	" "	Jakarta, Indonesia	1977
DEN-1	1236	"	" "	Jakarta, Indonesia	1978
DEN-1	1298	"	" "	Mexico	1980
DEN-1	1318	"	" "	Puerto Rico	1981
DEN-1	1335	"	" "	Colombo, Sri Lanka	1981
DEN-1	1344	"	" "	Mexico	1982
DEN-1	1351	"	" "	Colombia	1982
DEN-1	1378	"	" "	Mexico	1983
DEN-1	1412	"	" "	Mexico	1983
DEN-1	1413	"	" "	Haiti	1983
DEN-2	Bangkok 16803	Mosq. ?,C6/36 #1	" "	Bangkok, Thailand	1974
DEN-2	Indonesia 10410	" "	" "	Java, Indonesia	1973
DEN-2	JA-TVP-496	C6/36 #1	" "	Jamaica	Aug 1982
DEN-2	NC 9163	C6/36 #1	" "	New Caledonia	1972
DEN-2	Burma 40479	C6/36 #1	" "	Burma	1976
DEN-2	PM 33974	Mosq.#1,C6/36 #1	Aedes africanus	Rep. of Guinea	Nov 1981
DEN-2	PR-159	C6/36 #1	human serum	Puerto Rico	1969
DEN-2	Ph.H 2172	AP-61 #2,C6/36 #1	human serum	Manila, Philippines	1983
DEN-2	INS 348600	C6/36 #3	human serum	Tomaco, Narino, Colombia	Jan 1986
DEN-2	1232	Mosq. #2,C6/36 #2	human serum	Jakarta, Indonesia	1978
DEN-2	1251	"	" "	Tonga	1974
DEN-2	1268	"	" "	Jogyakarta, Indonesia	1978
DEN-2	1328	"	" "	Puerto Rico	1977

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
DEN-2	1329	Mosq. #1, C6/36 #2	human serum	Jamaica	1982
DEN-2	1334	"	"	Colombo, Sri Lanka	1981
DEN-2	1349	"	"	Upper Volta	1982
DEN-2	1353	"	"	Colombo, Sri Lanka	1982
DEN-2	1408	"	"	Jamaica	1983
DEN-2	1421	"	"	Mexico	1983
DEN-3	Burma DHF 190	Mosq?, C6/36 #1	"	Burma	1976
DEN-3	Tahiti 18	"	"	Tahiti	1964
DEN-3	PR-9311	"	"	Puerto Rico	1963
DEN-3	Thailand 49080	"	"	hailand	1971
DEN-3	Singapore 16182	"	"	Singapore	1973
DEN-3	1245	Mosq. #2, C6/36 #2	"	Sleman, Indonesia	1973
DEN-3	1259	"	"	Jakarta, Indonesia	1978
DEN-3	1301	"	"	Malaysia	1975
DEN-3	1309	LLC/MK$_2$#1,Mosq.#2,C6/36 #1	"	Bangkok, Thailand	1978
DEN-3	1325	Mosq. #2, C6/36 #1	"	Colombo, Sri Lanka	1981
DEN-3	1339	"	"	Puerto Rico	1977
DEN-3	1359	"	"	Colombo, Sri Lanka	1982
DEN-3	1363	"	"	Colombo, Sri Lanka	1982
DEN-3	083-144	C6/36 #1	"	Bangkok, Thailand	1988
DEN-4	1228	Mosq. #2, C6/36 #1	"	Jogyakarta, Indonesia	1978
DEN-4	1229	"	"	Jakarta, Indonesia	1976
DEN-4	1315	"	"	Puerto Rico	1981

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
DEN-4	1331	Mosq. #2, C6/36 #1	human serum	Puerto Rico	1982
DEN-4	1332	"	"	Puerto Rico	1982
DEN-4	1385	Vero #1, Mosq. #2, C6/36 #1	"	Boa Vista, Brazil	1982
DEN-4	1411	Mosq. #2, C6/36 #1	"	San Salvador, El Salvador	1983
DEN-4	1414	Mosq. #2, C6/36 #1	"	Mexico	1983
DEN-4	1415	"	"	Mexico	1983
DEN-4	Medan 12524	"	"	Sumatra, Indonesia	1973
DEN-4	Tahiti 79	"	"	Tahiti	1979
DEN-4	Sri Lanka	"	"	Sri Lanka	1978
DEN-4	Niue	"	"	Niue	1980
DEN-4	Gilberts 49367	"	"	Kiribati, Gilberts	1980
DEN-4	PR-TVP 376	C6/36 #1	"	Puerto Rico	Feb 1982
DEN-4	SH 38549	Mosq. #1, C6/36 #3	"	Dakar, Senegal	Nov 1983
DEN-4	38549	AP-61 #1, C6/36 #1	"	Dakar, Senegal	1977
DEN-4	38550	AP-61 #1, C6/36 #1	"	Dakar, Senegal	1980
EEE	ME-77132	Mosq. #1, C6/36 #1	Culiseta melanura	Carver Cdr Swmp. MA	Aug 1977
EEE	78-3372	C6/36 #1	"	Raynham, MA	Sep 1978
EEE	79-2138	C6/36 #1	"	Westport, MA	Aug 1979
EEE	DV-260-82	C6/36 #1	Parus bicolor	Dennisville, NJ	Jul 1982
EEE	VO80784/1	Vero #1	horse brain	Florida	1984
EEE	VO82085/2	Vero #1	horse brain	Florida	1985
EEE	44-84	Vero #1	bobwhite quail	Pocomoke Swamp, MD	1984

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
EEE	323-85	Vero #1	Culiseta melanura	Pocomoke Swamp	1985
EEE	187-85	Vero #1	"	"	1985
EEE	215-85	Vero #1	"	"	1985
EEE	GML 903866	Vero #3	sentinel chicken	Bayano, Panama	1984
EEE	GML 900188	SM #1, Vero #1	horse brain	Gatuncillo, Panama	196?
EEE	lung #72	Vero #1	whooping crane	Patuxent Wildlife, Research Center, MD	1984
EEE	FD #7829	Vero #2	Culiseta melanura	Pocomoke Cypress Swamp, MD	1983
EEE	FD #7830	Vero #2	"	Pocomoke Cypress, Swamp, MD	1983
EEE	Ma 2494	PCE #1, Vero #1	"	Raynham, MA	Sep 1977
EEE	Ma 2020	PCE #1, Vero #1	"	Halifax, MA	Aug 1978
EEE	Ma 1058	PCE #1, Vero #1	"	Halifax, MA	Jul 1979
EEE	Ma 1833	PCE #1, Vero #1	"	Halifax, MA	Aug 1980
EEE	Ma 396	PCE #1, Vero #1	"	New Bedford, MA	Aug 1982
EEE	Ma 843	PCE #1, Vero #1	"	Easton, MA	Sep 1983
EEE	Ma 1313	PCE #1, Vero #1	"	Raynham, MA	Sep 1984
EEE	MARU 435731	Vero #2	horse brain	Chepo, Panama	Jul 1986
EEE	M-210-33A	Original	horse brain	Colchester, CT	Sep 1983
EEE	MP-9	C6/36 #1	Culiseta melanura	Colchester, CT	Sep 1979
EEE	R-35108	C6/36 #1	horse brain	Three Rivers, MI	1980
EEE	70U1104	Vero #1	sentinel hamster	Iquitos, Peru	1970
EEE	M-649-84	Original	(horse brain)	Waterford, CT	1984

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
Highlands J	NJO-1111D	C6/36 #1	Culeseta melanura	S.R. Game Farm, NJ	1960
Highlands J	78-3331	C6/36 #1	Culiseta melanura	Canton, MA	Sep 1978
Highlands J	80-2138	C6/36 #1	"	Westport, MA	Aug 1979
Highlands J	WC-431	C6/36 #1	Dumetella carolinensis	West Creek, NJ	Sep 1981
Isfahan	01025-C	Vero #2	"	Isfahan, Iran	Aug 1975
Isfahan	01026-167	Vero #3	"	Isfahan, Iran	Aug 1975
Jamestown Canyon	MP-935	C6/36 #1	Aedes canadensis	Isfahan, Iran	1979
JE	Osaka 222681	Mosq., C6/36 #1	Culex tritaeniorhynchus	Osaka, Japan	1979
JE	Sagiyama 224052	"	"	Sagiyama, Japan	1956
JE	Java 222682	"	"	Java, Indonesia	1979
JE	HK 3256	Mosq #7, C6/36 #1	Culex annulus	Taiwan	197
JE	Ph.An 1242	Vero #1, C6/36 #1	pig blood	Santo Cristo, Philippines	1984
JE	KE-105/83	C6/36 #1	human brain	Kampangphet, Thailand	1983
JE	KE-094/83	C6/36 #1	human brain	Kampangphet, Thailand	1983
JE	B-1080/83	C6/36 #1	pig serum	Choomporn, Thailand	1983
JE	KP0035-114CT	C6/36 #2	Culex tritaeniorhynchus	Kampangphet, Thailand	1982
Karimabad	91015-AG	Vero #3	Phlebotomus papatasi	Isfahan, Iran	Aug 1975
Keystone	MB7-34EJ	Mosq #1, C6/36 #1	A. atlanticus-tormentus	Bay St. Louis, MS	Sep 1967
Keystone	FD-BHK	BHK #1, C6/36 #1	Aedes atlanticus	Pocomoke Swamp, MD	1975
Kokobera	CH 19620	Mosq #1, C6/36 #1	Culex annulirostris	Charleville, Aust.	Feb 1976
Junjin	CH 16532	C6/36 #2	Culex annulirostris	Charleville, Aust.	Mar 1974
La Crosse	78V 13193	SM #1, Vero #2	Aedes triseriatus (larvae)	North Carolina	1978
La Crosse	prototype	C6/36 #1	human brain	La Crosse, WI	1960
La Crosse	78134	C6/36 #1	human brain	Wisconsin	1978
Punta Toro	PaAr 2381	Vero #2	human brain	Bavano, Panama	Nov 1975
Punta Toro	Adames	Vero #3	man	Darien Pr., Panama	Apr 1972
Ross River	S-48325	C6/36 #4	human serum	Pago Pago, Am Samoa	Dec 1979
Ross River	Aus.Ar.90614	SM #1, C6/36 #1	Aedes sp.	New South Wales, Australia	1983

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
San Angelo	72V-4089	Mosq #2, C6/36 #1	Psorophora signipennis	Las Cruces, NM	1972
Sicilian	91025-B	Vero #3	"	Isfahan, Iran	1975
Sicilian	OSS-42	Vero #1	Phlebotomus sp.	Imbaba, Giza, Egypt	1986
Sicilian	91045-1	Vero #2	Phlebotomus papatasi	Isfahan Prov., Iran	Aug 1975
SLE	Ft. Wash.-4	Mosq #2, C6/36 #2	Culex pipiens	Ft. Washington, MD	Jan 1977
SLE	BFS-508	C6/36 #1	Culex tarsalis	Kern Co., CA	Aug 1950
SLE	Ft. Wash.-4	C6/36 #1	Culex pipiens	Pocomoke Swamp, MD	Jan 1977
SLE	FL 79-411	C6/36 #1	Culex nigripalpus	Lee County, Florida	1979
SLE	BFS-2874	C6/36 #1	Culex tarsalis	Kern Co., CA	Sep 1960
SLE	BFN-1324	C6/36 #1	Culex tarsalis	Butte Co., CA	Jul 1970
SLE	E-2819	C6/36 #1	Culex tarsalis	Riverside Co., CA	Jul 1980
Snowshoe hare	82-Y-21	C6/36 #1	Aedes nigripes	Yukon Ter., Canada	Jul 1982
VEE (1-D)	71D 1394	SM #1, C6/36 #1	Mixed mosquitoes	Peru	1971
VEE (1-D)	310979	C6/36 #1	Sentinel hamster	Puerto Boyaca, Colombia	Jun 1974
VEE (1-E)	68U 200	C6/36 #1	Sentinel hamster	Avellana, Guatemala	May 1977
VEE	64A 87	SM #1, C6/36 #1	Culex opisthopus	Sontecomapan, Mexico	1964
VEE	64U 60	SM #1	sentinel hamster	Santecomapan, Mexico	1964
VEE	83U 12	SM #1	sentinel hamster	Rio de Oro, Colombia	1983
VEE-IC	CBS1-9	Vero #2	Anopheles triannulatus	Sotillo, Venezuela	1963
VEE-IE	68U 201	Vero #1	sentinel hamster	La Avellana, Guatemala	1968
VEE-ID-E	71D 1249	SM #1, Vero #1	Mosquito pool	Iquitos, Peru	1971
VEE-ID-E	70U 1139	Vero #2	sentinel hamster	Iquitos, Peru	1970
VEE-IE	68U 201	SM #1, Vero #1	sentinel hamster	La Avellana, Guatamala	1968
VEE-IE	71U 338	SM #1, Vero #1	sentinel hamster	Monte Rico, Guatemala	1971
VEE-V	GaAr 4389	SM #4, CEC #1, Vero #1	Culex spp. (pool)	Cabassou, French Guyana	1974

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
VEE-III	TVL 52049	Vero #2	Zygodontomys brevicauda	Bush-Bush, Trinidad	1963
VEE-IB	541/73	SM #1, CEC #2, Vero #1	human	Guajira, Venezuela	1973
VEE-IA	E1/68	SM #1, CEC #1, Vero #1	human	Guajira, Venezuela	1968
VEE-ID	202330	SM #1, Vero #2	Proechimys semispinosus	Gamboa, Panama	1963
VEE-IC	V-198	SM #1, Vero #2	human serum	Guajira, Colombia	1962
VEE-IC	V-178	SM #1, Vero #2	human	Cundinamarca, Colombia	1961
VEE-IE	63A 216	SM #2, Vero #1	Culex spp. (pool)	Sontecomapan, Mexico	1963
VEE-ID	3880	SM #2, Vero #3	human serum	Canito, Panama	1961
VEE-III	Fe 37C	SM #6, Vero #1	Culex spp. (pool)	Florida, USA	1963
VEE-ID-E	Tumaco An9004	SM #3, Vero #1	sentinel hamster	Pacific coast, Colombia	1969
VEE-IE	71U 384	SM #1, Vero #1	sentinel hamster	La Avellana, Guatemala	1971
VEE (IAB)	G 8419	SM #2, Vero #2	horse	Sonora, Mexico	1972
VEE-IA	71D 1252	SM #1, Vero #1	Mosquito pool	Iquitos, Peru	1971
VEE-IA	E123/69	SM #1, CEC #1, Vero #1	human	Mara, Venezuela	1969
VEE-IB	69U332	Vero #1	sentinel hamster	La Avellana, Guatemala	1969
VEE-ID	V-209A	SM #2, Vero #1, Vero #2	sentinel mouse, bov. teat	Alta Verapaz, Guatemala	1986
VEE-IV	BeAr 35645	SM #4, Vero #1	Anopheles nimbus	Belem, Brazil	1961
VEE-IA	52/73	SM #2, Vero #1	burro	La Libertad, Peru	1973
VEE-II	Fe5-47 et	SM #2, Vero #1	Aedes taeniorhynchus	Florida, USA	1965
VEE-IB	6921	SM #2, Vero #1	human serum	Montufar, Guatemala	1969
VEE-ID-E	CoAn 59145	? Vero #1	sentinel hamster	Santander, Colombia	1970
VEE-IA	CoAn 5384	SM #2, CEC #1, Vero #1	horse	Carmelo, Colombia	1967
VEE-V	CoAn 4104	SM #3, Vero #1	Psaracolus decumanus	Tonate, French Guyana	1973
VEE-IA	71D 1252	SM #1, Vero #1	Mosquito pool	Iquitos, Peru	1971
VEE-ID-E	75D 143	SM #2, Vero #1	Mosquito pool	Iquitos, Peru	1975
VEE-IV	BeAr 40403	SM #6, Vero #1	Trichoprosopon digitatum	Belem, Brazil	1961

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
VSV-IND	L53-85	Vero #1	bovine mouth	Cartago, Costa Rica	1985
VSV-IND	L28-87	Vero #1	bovine mouth	Sta. Ana, San Salvador	1987
VSV-IND	L274-86	Vero #1	bovine mouth	Chalatenango, San Salvador	1986
VSV-IND	L96-87	Vero #1	bovine teat	Alajuela, Costa Rica	1987
VSV-IND	GML 903816	Vero #4	human (throat swab)	Panama City, Panama	1984
VSV-IND	L5-85	Vero #1	bovine teat	Guatemala	1984
VSV-IND	L30-84	Vero #1	bovine mouth	Costa Rica	1984
VSV-IND	L125-84	Vero #1	bovine teat	Panama	1984
VSV-IND	L2-83	?, Vero #1	"	Honduras	1983
VSV-IND	L51-85	Vero #1	"	Costa Rica	1985
VSV-IND	L111-85	Vero #1	"	Salvador	1985
VSV-N.J.	Jardin 12-IV	bovine #1, Vero #1		Veracruz, Mexico	1982
VSV-N.J.	Ossabaw	Vero #2	feral pig	Ossabaw Island, Georgia	1983
VSV-NJ	L32-85	Vero #1	bovine epithelium	Managua, Nicaragua	1985
VSV-NJ	L7-82	Vero #2	bovine epithelium	Honduras	1982
VSV-NJ	L11-85	Vero #1	bovine teat	Panama	1985
VSV-NJ	L14-85	Vero #1	bovine teat	Costa Rica	1984
VSV-NJ	L35-85	Vero #1	bovine mouth	Nicaragua	1985
VSV-NJ	L264-84	Vero #1	bovine teat	Honduras	1983
VSV-NJ	82A175	Vero #1	Equus caballus	Loveland, Colorado	Sep 1982
VSV-NJ	L270-84	Vero #1	bovine mouth	Guatemala	1984
VSV-NJ	L243-84	Vero #1	bovine mouth	Belize	1984
VSV-NJ	L8-82	Vero #1	bovine epithelium	Honduras	1982
VSV-NJ	L14-82	Vero #1	"	Costa Rica	1982
VSV-NJ	L67-82	Vero #1	"	Guatemala	1982
VSV-NJ	L153-83	Vero #1	"	Nicaragua	1983

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
VSV-NJ	L183-83	Vero #1	bovine epithelium	Panama	1983
VSV-NJ	L177-85	Vero #1	bovine epithelium	Honduras	1985
VSV-NJ	L172-86	Vero #1	bovine mouth	Managua, Nicaragua	1986
VSV-NJ	L123-87	Vero #1	bovine mouth	Managua, Nicaragua	1987
VSV-NJ	L44-37	Vero #1	bovine mouth	Alta Verapaz, Guatemala	1987
VSV-NJ	L130-87	Vero #1	bovine mouth	Comayagua, Honduras	1987
VSV-NJ	L212-86	Vero #1	bovine mouth	Chiriqui, Panama	1986
VSV-NJ	L204-86	Vero #1	bovine teat	Alta Verapaz, Guatemala	1986
WEE	NM5-7ET	C6/36 #1	Aedes vexans	Rancho de Albiquin, NM	1965
WEE	BFS-1428	C6/36 #1	Culex tarsalis	Kern Co., CA	Jun 1952
WEE	DLAN-23-82	Cy/36 #1	Culex tarsalis	Tulane Co., CA	Jul 1982
WEE	BFS-4143	C6/36 #1	Culex tarsalis	Kern Co., CA	Jul 1962
WEE	BFN-3258	C6/36 #1	Culex tarsalis	Glenn Co., CA	Aug 1971
YF	1362/77	C6/36 #1	human serum	Mutucana, Peru	1977
YF	5384	SM #2, CEC #1	horse	Carmelo, Colombia	1967
YF			human serum	Antioquia, Colombia	1985
YF	HD 38564	C6/36 #?	human serum	Upper Volta	1982
YF	Ar B 9005	SM_5, $MOSQ_1$	Aedes africanus	Bozo, Cent. Afr. Rep.	Nov 1977
YF	Ar B 8883	SM_5, $MOSQ_1$	Aedes africanus	Bozo, Cent. Afr. Rep.	Oct 1977
YF	$P128M_c$ SMB/IVIC	SM_5, $MOSQ_1$	liver of Alouatta seniculus	Las Claveles, Cojedes, Venezuela	Oct 1959
YF	INS-347613	C6/36 #3	human serum	Antioquia, Colombia	1985
YF	11117505	SM #4	human serum	Oghomosho, Nigeria	4/22/87
YF	11117491	SM #4	human serum	Oghomosho, Nigeria	2/26/87
YF	BA55	SM #3	human serum	Oghomosho, Nigeria	5/23/87

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
YF	PHO-42H, SMB/IVIC-2	SM_2, $MOSQ_1$	human liver	San Rafael de el Pinal, Tachira, Venezuela	Nov 1961
YF	Asibi	Monkey 4	human serum	Kpeve, Ghana	Jun 1927
YF	R35740	SM1, Mosq. 1	human liver	Ayacucho Dept., Peru	Feb 1979
YF	Jiminez	*Aotus* 1	human liver	Panama	1974
YF	Serie	SM 7, Mosq. 1	?	Ethiopia	1961
YF	V-528A	*Alouatta* 1, Mosq. 1	human serum	Colombia	1979
YF	14-FA	SM 7, Mosq. 1	human serum	Luanda, Angola	1971
YF	E-1337	SM 1, Mosq. 1	human serum	Guayzimi, Ecuador	1979
YF	PM 27340	Mosq. 3	*Aedes furcifer-taylori*	Kedougou, Senegal	1978
YF	M 37	Mosq. 3	human serum	Toubacouta, Senegal (patient died in Paris)	1979
YF	Ar B 11059	AP-61 #1, C6/36 #1	*Aedes africanus*	Bozo, Cent. Afr. Rep.	1977
YF	Ar B 17239	AP-61 #1, C6/36 #1	*Aedes africanus*	Bozo, Cent. Afr. Rep.	1980
YF	SH 38556	AP-61 #1, C6/36 #1	human blood	Burkina Faso	1983
YF	Ar D 25865	SM 5, Mosq. #1	*Aedes furcifer-taylori* (males)	Kedougou, Senegal	1977
YF	T 797984	Mosq. 1	human liver	Trinidad	1979
YF	T 790882	Mosq. 1	*Haemagogus janthinomys*	Trinidad	1979
YF	Ar 232869	SM 2, Mosq. 1	*Haemagogus* sp.	Brazil	Mar 1973
YF	Ar 350397	SM 2, Mosq. 1	*Haemagogus* sp.	Belterra, Para, Brazil	Aug 1978
YF	H 3509698	SM 2, Mosq. 1	human liver	Tome-Acu, Para, Brazil	Aug 1978
YF	Be Ar 233164	Mosq. 4	*Haemagogus* sp.	Goias, Brazil	1973

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
YF	TRVL 4205	Actus$_1$, MOSQ$_2$	liver of dead *Alouatta seniculus*	Trinidad	1954
YF	79 H 327	MOSQ$_2$	human serum	Minteh Kunda, N. Bank Div., Gambia	Jan 1979
YF	38578	AP-61 #1, C6/36 #1	human blood	Burkina Faso	1983
YF	38580	AP-61 #1, C6/36 #1	human liver	Burkina Faso	1983
YF	38581	AP-61 #1, C6/36 #1	human liver	Burkina Faso	1983
YF	38557	AP-61 #1, C6/36 #1	human liver	Burkina Faso	1983
YF	38558	AP-61 #1, C6/36 #1	human liver	Burkina Faso	1983
YF	38564	AP-61 #1, C6/36 #1	human liver	Burkina Faso	1983
YF	38566	AP-61 #1, C6/36 #1	human liver	Burkina Faso	1983
YF	38570	AP-61 #1, C6/36 #1	human liver	Burkina Faso	1983
YF	38571	AP-61 #1, C6/36 #1	human liver	Burkina Faso	1983
YF	38572	AP-61 #1, C6/36 #1	human liver	Burkina Faso	1983
YF	38574	AP-61 #1, C6/36 #1	human liver	Burkina Faso	1983
YF	38576	AP-61 #1, C6/36 #1	human liver	Burkina Faso	1983
YF	38577	AP-61 #1, C6/36 #1	human liver	Burkina Faso	1983
YF	38329	AP-61 #1, C6/36 #1	Ae. furcifer	Burkina Faso	1983
YF	38334	AP-61 #1, C6/36 #1	Ae. furcifer	Burkina Faso	1983
YF	38335	AP-61 #1, C6/36 #1	Ae. furcifer	Burkina Faso	1983
YF	38390	AP-61 #1, C6/36 #1	Ae. furcifer	Burkina Faso	1983
YF	38400	AP-61 #1, C6/36 #1	Ae. furcifer	Burkina Faso	1983
YF	38087	AP-61 #1, C6/36 #1	Ae. furcifer	Kedougou, Senegal	1983
YF	38088	AP-61 #1, C6/36 #1	Ae. furcifer	Kedougou, Senegal	1983
YF	38089	AP-61 #1, C6/36 #1	Ae. taylori	Kedougou, Senegal	1983
YF	31104	AP-61 #1, C6/36 #1	Ae. furcifer	Kedougou, Senegal	1983

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality	Date
YF	38693	AP-61 #1,C6/36 #1	Ae. furcifer & africanus	Kedougou, Senegal	1983
YF	37961	AP-61 #1,C6/36 #1	Ae. furcifer	Kedougou, Senegal	1983
YF	M-37	MOSQ #2, C6/36 #1	Human serum	Kedougou, Senegal	Nov 1979
YF	PM 27340	MOSQ #2, C6/36 #1	Ae. furcifer-taylori	Kedougou, Senegal	Oct 1978
YF	Ar B 8883	SM_5, $MOSQ_1$	Ae. africanus	Bozo, Cent.Afr.Rep.	Oct 1977
YF	Ar D 25865	SM_5, $MOSQ_1$	Ae. furcifer-taylori (males)	Kedougou, Senegal	Dec 1977
YF	T 797984	$MOSQ_1$	human liver	Trinidad	1979
YF	T 790882	$MOSQ_1$	Haemagogus janthinomys	Trinidad	1979
YF	Ar 232869	SM_2, $MOSQ_1$	Haemagogus sp.	Brazil	Mar 1973
YF	Ar 350397	SM_2, $MOSQ_1$	Haemagogus sp.	Belterra,Para,Brazil	Aug 1978
YF	H 3509698	SM_2, $MOSQ_1$	human liver	Tome-Acu,Para,Brazil	Aug 1978
YF	Be Ar 233164	$MOSQ_4$	Haemagogus sp.	Goias, Brazil	1973
YF	TRVL 4205	$Aotus_1$, $MOSQ_2$	liver of dead Alouatta seniculus	Trinidad	1954
YF	79 H 327	$MOSQ_2$	human serum	Minteh Kunda,N.Bank Div., Gambia	1979
YF	F128M_c SMB/IVIC	SM_3, $MOSQ_1$	liver of Alouatta seniculus	Las Claveles, Cojedes, Venezuela	Oct 1959
YF	PHO-42H,SMB/IVIC-2	SM_2, $MOSQ_1$	human liver	San Rafael de el Pinal,Tachira,Venezuela	Nov 1961
YF	Asibi	Monkey 4	human liver	Kpeve, Ghana	Jun 1972
YF	R35749	SM_1, $MOSQ_1$	human liver	Avacucho Dept., Peru	Feb 1979
YF	Jiminez	$Aotus_1$	human liver	Panama	1974
YF	Serie	SM_7, $MOSQ_1$	?	Ethiopia	1961

SCLAS Low Passage Virus Strains

Virus	Strain	Passage	Source	Locality
YF	V-528A	Alouatta 1, Mosq.1	human serum	Colombia
YF	14 FA	SM 7, Mosq.1	human serum	Luanda, Angola
YF	E-1337	SM 7, Mosq. 2	human serum	Guayzimi, Ecuador
YF	PM 27340	Mosq. 3	Aedes furcifer-taylori	Kedougou, Senegal
YF	M 37	Mosq. 3	human serum	Toubacouta, Senegal (patient died in Paris)
Zika	41662	AP-61 #1, C6/36 #1	Ae. taylori	Kedougou, Senegal
Zika	41667	AP-61 #1, C6/36 #1	Ae. taylori	Kedougou, Senegal
Zika	41671	AP-61 #1, C6/36 #1	Ae. taylori	Kedougou, Senegal
Zika	41519	AP-61 #1, C6/36 #1	Ae. taylori	Kedougou, Senegal
Zika	41524	AP-61 #1, C6/36 #1	Ae. taylori	Kedougou, Senegal
Zika	41525	AP-61 #1, C6/36 #1	Ae. taylori	Kedougou, Senegal

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